The DREAM complex: Master coordinator of cell cycle dependent gene expression

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Preface

The dimerization partner (DP), retinoblastoma (RB)-like, E2F and MuvB (DREAM) complex provides a previously unsuspected unifying role in the cell cycle by directly linking p130, p107, E2F, BMYB and FOXM1. DREAM mediates gene repression during G0 and coordinates periodic gene expression with peaks during G1/S and G2/M. Perturbations in DREAM regulation shift the balance from quiescence towards proliferation and contribute to increased mitotic gene expression levels frequently observed in cancers with poor prognosis.

Cell cycle regulated gene expression is highly coordinated within and between each phase of the cell cycle. The coordinated periodic expression of hundreds of genes with peaks during G1/S and G2/M in human cells was recognized 10 years ago [1]. On receiving appropriate growth factor signalling, a quiescent cell will enter the cell cycle, pass the restriction point and, during the G1 to S phase transition, turn on genes required for DNA synthesis (Figure 1A). The control of the cell cycle by RB, the product of the retinoblastoma tumour suppressor gene (*RB1*), is well established (Box 1). However, the control of entry into S phase by RB does not fully account for how gene expression is regulated throughout the cell cycle. For example, it was never understood how RB regulated the expression of genes, such as cyclin B1 (*CCNB1*), or hundreds of other late cell cycle genes, whose mRNA levels peak during G2/M and whose protein products are required to complete mitosis. In contrast to the cyclical waves of gene expression present in cycling cells, expression of nearly all genes required for DNA synthesis and mitosis are reduced when cells exit the cell cycle and enter into the quiescent or G0 phase in response to differentiation signals or absence of growth factors. The ability of RB to promote or maintain entry into the quiescent or G0 phase of the cell cycle was also never a specific part of the RB cell cycle model as illustrated in Figure 1A. Thus, there has been indirect evidence for additional cell cycle regulatory pathways that are not reliant on RB.

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Recently, several unexpected discoveries revealed that the RB-like p130 (*RBL2*) and p107 (*RBL1*), together with BMYB (*MYBL2*) and Forkhead box M1 (FOXM1), coordinate cell cycle dependent gene expression through a common pathway. p130 or p107 forms a multi-subunit protein complex containing dimerization partner (DP), RB-like, E2F and MuvB (DREAM) that represses most if not all cell cycle gene expression during quiescence. The MuvB core component of the DREAM complex also coordinates gene expression during S phase and G2/M through its interactions with BMYB and FOXM1 (Figure 1B). The function of the highly conserved DREAM complex — components are conserved in vertebrates, flies and worms — is the focus of study by many laboratories.

Disruption of the cell cycle is a well-known occurrence in cancer and many of the genes involved in cell cycle regulation, such as *MYC*, cyclin D1 (*CCND1*) and *RB1*, are known to be deregulated, mutated, amplified or lost during tumour progression. Because of these perturbations, understanding the coordination of gene expression during the cell cycle has been a focus of cancer research for many years. Moreover, since BMYB and FOXM1 are overexpressed in a number of tumour types, understanding the function of the DREAM complex and the MuvB core during tumorigenesis has become an area of research focus. In this Review, we discuss DREAM and the MuvB core, their known functions within the cell cycle and the relevance this has for cancer research.

MuvB genes and protein complexes

Caenorhabditis elegans and the synMuv genes

The laboratory of Robert Horvitz identified a signalling pathway in *Caenorhabditis elegans* that controlled vulva development[2] (Figure 2A). Loss-of-function mutations in the Vul class of genes led to the absence of a vulva[2]. Cloning of the Vul genes revealed that they were involved in cellular proliferation and included homologues of EGF, EGFR and RAS[3-5]. Increased activation of the Vul genes results in multiple vulva type organs referred to as the multi-vulva phenotype (Muv). In addition, certain combinations of loss-of-function mutant genes also resulted in worms with the Muv phenotype[6]. These genes were termed synthetic multi-vulva (synMuv). Three classes of synMuv genes, A, B and C, emerged all of which opposed the receptor tyrosine kinase-RAS signalling cascade required for normal vulva development [7]. While the class A synMuv genes appear to be involved in regulating EGF expression, the class B synMuv genes corresponded to the worm homologues of RB (Lin-35), E2F (EFL-1 and EFL-2) and DP (DPL-1) (Figure 2A) [89]. In addition, the class B synMuv genes included several genes, Lin-54, Lin-53, Lin-37, Lin-9 and Lin-52, with unknown function [1011]. The B class also contained components of the NuRD (Nucleosome Remodeling and Deacetylation) complex and the C class contained homologues of the Tip60–TRAPP complex [12] indicating that the regulation of chromatin and therefore gene expression was likely to be important in the generation of the Muv phenotype in *C. elegans*.

Purification of the fly dREAM and MMB complexes

Biochemical studies in flies brought further insight into the function of the uncharacterized worm synMuvB genes. In ovarian follicle cells, repeated rounds of DNA replication results in gene amplification of the chorion locus [13]. The Botchan laboratory purified a protein

complex that could bind specifically to this locus. The complex contained Myb plus four proteins named Myb interacting proteins (Mip): Mip130 (a homologue of LIN9); Mip120 (a homologue of LIN54); Mip40 (a homologue of LIN37); and Caf1 (also known as p55, a homologue of RBBP4) (Figure 2B) [14]. Because the Mips had strong homology to the previously uncharacterized worm synMuvB genes, the complex was named Myb-MuvB (MMB). Purification of the native Mip120 and epitope-tagged Mip130 confirmed the presence of Myb, Mip130, Mip40 and Caf1 and also revealed Lin52 and the RB homologues Rbf1, Rbf2, E2f2 and Dp in the MMB complex [15]. Mutation of Mip130 or RNA interference (RNAi)-mediated knockdown of Mip130, Mip120 or Caf1 led to decreased chorion gene amplification confirming the role of the MMB complex in this process [16].

In a separate study, a collaborative effort between the Brehm and Dyson laboratories focused on identifying proteins bound to Rbf and E2f in drosophila embryo nuclear extracts [17]. They purified a complex containing Rbf1, Rbf2, Dp, E2f2, Myb and several homologues of the synMuvB genes including Mip130, Mip120, Mip40 and Caf1 and named it the drosophila RBF, E2F2 and Mip (dREAM) complex [17]. A role for the dREAM complex in gene repression was supported by the observation that RNAi-mediated knockdown of E2f2, Mip130 or Mip120 led to increased expression of E2F target genes that were typically silenced in Drosophila melanogaster cell lines (Figure 2A) [18,17,15,19].

Following identification of the fly MMB and dREAM complexes, a similar complex was immuno-purified from C. *elegans* extracts and named DP, RB and MuvB (DRM). The worm DRM complex contained LIN-9, LIN-35 (RB), LIN-37, LIN-52, LIN-53 (RBBP4), LIN-54 and DPL-1 [20]. Notably, Myb was not purified with the worm DRM complex [7, 20] that may reflect the absence of an obvious MYB homologue in *C. elegans* [21].

Mammalian DREAM and BMYB-MuvB complexes

Identification of the mammalian DREAM complex followed the discoveries of the fly MMB and dREAM and worm DRM complexes. The homologous LIN9 protein was recognized in silico and found to cooperate with RB in cellular growth suppression assays [22, 23]. Immunoprecipitation of the RB-like protein p130 followed by mass spectrometry based identification of associated proteins showed that mammalian cells also contained a large multi-protein complex that consisted of E2F4 or E2F5 and their heterodimeric DP partners, as well as LIN9, LIN54, LIN37, LIN52 and RBBP4 that comprise the MuvB core [24] (Figure 2B).

Notably, the mammalian p130-containing DREAM complex does not contain a MYB family protein. Instead, immunopurification of individual MuvB core factors followed by mass-spectrometry detected the MuvB core, p130, p107, BMYB and AMYB (MYBL1) [24, 25]. Furthermore, immunoprecipitation of BMYB, a sequence specific DNA binding factor, co-precipitated all 5 MuvB core proteins but not p130, p107, E2F4 or DP1. This suggested that the mammalian MuvB core binds to either p130 or p107 to form the DREAM complex or to BMYB to form the BMYB–MuvB complex. These two distinct complexes contrast with the fly MMB complex that contains both RB and MYB together with the MuvB core (Figure 1B, Table 1).

The p130-containing DREAM complex is present in serum-starved or quiescent G0 cells, whereas the BMYB-MuvB complex is found in S phase enriched cell populations [24-26]. Although several labs have shown that p107 can bind to the MuvB core, the exact cell types or conditions when it binds have not been clearly defined [24-26]. Similarly, AMYB can bind to the MuvB core although this may occur in specific cell types or tissues [24]. Earlier studies which reported that RB binds to LIN9 or p107 binds to BMYB may have reflected results obtained with transiently expressed plasmids[22, 27]. Instead RB appears to perform functions distinct from the DREAM complex and p107 binds the MuvB core without BMYB [22, 24, 28, 29].

DREAM controls quiescence

DREAM binds E2F regulated gene promoters in quiescence

The RB-like protein p130 is typically expressed in quiescent mammalian cells. The high levels of p130 expression during quiescence and its ability to bind to the repressor E2F4 and E2F5 transcription factors suggested a role for p130 in repression of E2F dependent transcription. Indeed, repression of cell cycle gene promoters in quiescent cells is associated with recruitment of E2F4 and p130 as well as low levels of histone acetylation [30]. By contrast, levels of p130 are kept low in proliferating cells due to ubiquitin-mediated degradation [31].

Genome-wide chromatin immunoprecipitation (ChIP) studies of the mammalian DREAM complex provided clues into the scope of its activity. ChIP with antibodies specific for p130, E2F4, LIN9 and LIN54 followed by microarray analysis (ChIP-chip) found a significant overlap of binding enrichment for each factor at hundreds of sites across the genome [24]. As expected, the binding sites identified for p130 overlapped to a large degree with those detected for E2F4, but they also significantly overlapped with binding sites for LIN9 and LIN54. Enrichment for one factor at any site was accompanied by similar enrichment for the other components of the DREAM complex. This result implies that p130 and E2F4 bind to promoters together with the MuvB core as an intact DREAM complex. Location analysis and gene ontology analysis revealed that these binding sites were positioned close to the transcription start site of hundreds of genes whose levels change during the cell cycle. Notably, genes with peak levels in early (G1/S) or late (G2/M) phases of the cell cycle were included among the DREAM targets. In contrast, ChIP studies of the fly dREAM/MMB complex in Drosophila cell lines revealed a much broader occupancy; close to a third of all promoters were bound by members of this complex[32]. This may reflect a role for the fly dREAM/MMB complex in gene regulation beyond the cell cycle.

Disruption of various components of the DREAM complex by mutation or RNAi led to the loss of cell cycle dependent gene repression in G0. Normally, p107 is only expressed in proliferating cells and not during quiescence. However, knock down or mutation of p130 results in the increased expression of p107 [24, 33] and formation of a p107-containing DREAM complex in serum-starved, quiescent cells. However, if the levels of p107 and p130 are simultaneously reduced by knockdown or knockout, then the MuvB core was unable to form a functional DREAM complex during quiescence and repression of E2F-dependent genes was lost [24, 29]. The result is consistent with the observation that entry into

the cell cycle from G0 occurred earlier in *p107−/−;p130−/−* double knock out MEFs than in wild type cells and genes such as *Mybl2* and *Rrm2* were deregulated in these knockout MEFs[33]. In serum-starved, quiescent cells, RNAi-knockdown of LIN9 or the combined loss of p130 and p107 also led to increased cell cycle gene expression[24]. In contrast however, *Lin9* mutant MEFs arrested normally upon serum starvation and entered into S phase with normal kinetics after serum stimulation[34]. E2F4 and E2F5 may also complement each other in the DREAM complex. For example, *E2f4−/−;E2f5−/−* double knockout MEFs re-entered the cell cycle from G0 with normal kinetics but failed to arrest in G1 in response to p16INK4A (CDKN2A) [35].

The DREAM complex binds to at least two distinct DNA elements in the promoters of cell cycle dependent genes (see also Table 2). Through p130, E2F4 and DP1, the DREAM complex binds to E2F binding sites [24]. Similar to the E2F transcription factors themselves, E2F binding sites have been separated into activator and repressor sites. Although it is not always possible to distinguish between them, distinct repressor and activator E2F binding sites have been identified for a few cell cycle dependent gene promoters [36]. In addition to E2F, the MuvB core functions as a sequence specific DNA binding factor. It has been known for many years that several genes expressed during late S or G2/M phase contain a common sequence in their promoter regions known as the cell cycle genes homology region (CHR) element [37]. A DNA-affinity column purification of proteins that bind CHR identified several subunits of the MuvB core complex [38]. Specifically, the LIN54 subunit binds directly to the CHR element in promoters of genes, such as *CDK1* (also known as *CDC2*)[38, 39]. For at least some promoters, CDE sites, which may represent special E2F-binding elements, and the CHR elements are in close proximity to each other [37]. An element similar to CHR was found closely situated to E2F sites in the BMYB promoter and referred to as the downstream repression site (DRS) and likely represents a MuvB binding site as well [40, 41]. Given their proximity and the ability of p130–E2F4 to form a stable complex with the MuvB core, it is likely that the specificity and binding affinity of the DREAM complex to promoters is increased when the E2F and MuvB components of the DREAM complex cooperate in binding to more than one sequence element.

DYRK1A-dependent DREAM assembly

Different signals control the assembly of the DREAM complex and entry into the quiescent phase. Several components of the DREAM complex have been shown to be phosphorylated in cells. In particular, LIN52, the smallest subunit of the MuvB core, had a strikingly variable phosphorylation pattern that was dependent on its interacting partners. When co-immunoprecipitated by p130, LIN52 was always phosphorylated on the serine 28 (S28) residue [29]. By contrast, both phosphorylated and non-phosphorylated forms of S28-LIN52 were found in immuno-complexes of other components of the MuvB core or with BMYB. Alanine substitution of S28 (S28A-LIN52) did not affect the binding of LIN52 to other components of the MuvB core or to BMYB, but it reduced the binding of the MuvB core to p130 [29]. In addition, expression of the S28A-LIN52 mutant reduced the ability of cells to enter quiescence and cells continued to proliferate in response to serum starvation [29]. These results indicate that an intact Serine 28 residue in LIN52 was required for p130 binding to the MuvB core and DREAM assembly.

The dual specificity tyrosine (Y)-phosphorylation-regulated kinase 1A (DYRK1A) contributes to phosphorylation of S28-LIN52. Loss or inhibition of DYRK1A reduced the levels of phosphorylated S28-LIN52 and p130 binding to the MuvB core, thereby eliminating the DREAM complex (Figure 2B and 2C) [29]. The DYRK subfamily of protein kinases in mammals has five members [42]. Both DYRK1A and the closely related DYRK1B could phosphorylate S28-LIN52 in vitro while RNAi-knockdown of DYRK1A reduced levels of S28-LIN52 phosphorylation and DREAM complex (Figure 2B and 2C)[29]. Consequently, loss of DYRK1A activity would be predicted to compromise quiescence and increase the expression of E2F dependent genes. DYRK1A catalytic activity directed towards LIN52 can be regulated by the large tumour suppressor 1 (LATS1) or LATS2 kinases [43]. Knockdown of LATS1 or LATS2 reduced the ability of DYRK1A to phosphorylate S28-LIN52, compromised p130 binding to the MuvB core and reduced the ability of cells to enter quiescence and senescence [43]. LATS1 and LATS2 are downstream effector kinases in the Hippo tumour suppressor pathway that can phosphorylate the transcriptional co-activator, Yes-associated protein 1 (YAP1) [44, 4546]. Phosphorylation of YAP1 reduces its ability to activate TEA domain protein 1 (TEAD1), a transcriptional coactivator that promotes cellular proliferation[47-50]. Therefore, decreased LATS1 and LATS2 activity, and perhaps reduced Hippo signalling, would reduce DYRK1A-mediated DREAM complex assembly and entry into quiescence while permitting YAP1 to activate the TEAD proteins to facilitate cell growth.

DREAM and senescence

The DREAM complex is sensitive to disruption by the same growth promoting and oncogenic stimuli as RB (Figure 1A). For example, cyclin D–CDK4 can disrupt the DREAM complex by phosphorylation of p130 releasing it from E2F4–DP1 and LIN9. The N-terminal 84 residues of LIN9 are required for binding to p107 or p130 [51]. Expression of a LIN9 mutant lacking this N-terminal region (LIN9-Delta84) bypasses the requirement for CDK4 activity, resulting in increased levels of E2F target genes when CDK4 is inhibited [51]. Viral oncoproteins, such as HPV E7 and SV40 large T antigen, can also disrupt the DREAM complex. For example, the levels of the DREAM complex were decreased in the HPV-transformed cervical carcinoma cell lines CaSki and SiHa [52]. RNAi mediated knockdown of HPV E7 restored DREAM complex formation and induced quiescence in these cells. Importantly, although the DREAM complex was also not detected in HeLa cells, the MuvB core complex remained intact and was capable of binding to BMYB [53]. Therefore, viral oncogene binding to p130 disrupts binding to E2F4, DP1 and the MuvB core but does not appear to interfere with BMYB binding to the MuvB core.

Despite these similarities to RB, the DREAM complex may serve a fundamentally distinct role in establishing and maintaining oncogene-induced senescence. Senescence is thought to have a tumour suppressive function through its engagement as a result of the deregulation of certain oncogenes, such as members of the RAS family [54, 55]. DYRK1A-dependent phosphorylation of S28-LIN52 and recruitment of p130 to the MuvB core to form the DREAM complex is required for RAS-induced senescence in human BJ diploid fibroblasts immortalized by over-expression of telomerase reverse transcriptase (TERT) [29]. Inhibition of DYRK1A kinase activity or expression of S28A-LIN52 can suppress RAS-G12V-

induced senescence in these cells. By contrast, Lowe and colleagues found that RNAi mediated knockdown of RB alone but not p107 and p130 could partially overcome HRAS-G12V-induced senescence in the human diploid fibroblast IMR90 cell strain [28]. Although these studies are supportive of the model that RB and the DREAM complex provide distinct functions during senescence, the differences in these studies may reflect differences in the choice of cells. IMR90 cells have a strong dependence on the RB and p53 pathways for the induction of senescence. By contrast, BJ cells have less prominent p16^{INK4a} (CDKN2A) expression compared with IMR90 or MRC5 cells perhaps making them somewhat less sensitive to the RB pathway [56]. Studies in mice or specific tissues may bring further insight into the different roles of RB and DREAM.

The switch from DREAM to BMYB-MuvB

When cells leave G0 and enter into the cell cycle, p130 is dissociated from the repressor E2Fs (E2F4 and E2F5) and the MuvB core resulting in the release of the DREAM complex from cell cycle regulated promoters [24-26, 53, 57]. Activator E2Fs (E2F1, E2F2 and E2F3) are recruited to the promoters of genes with peak expression during the G1 to S phase transition [30] (Figure 3A). These early cell cycle genes encode many factors required for DNA synthesis and are a subset of the genes bound by the DREAM complex during quiescence. BMYB is among the many genes repressed by the DREAM complex during quiescence and subsequently expressed during the G1/S transition [24, 58, 59].

BMYB has long been known to bind to the promoters of G2/M genes such as *CDK1* and *CCNB1* and activating their transcription[36]. However, recently it was demonstrated that BMYB binding to late cell cycle promoters is dependent on its interaction with the MuvB core and vice versa. For example, RNAi-mediated knockdown of BMYB or the MuvB core reduced binding of both factors to late cell cycle gene promoters [53]. Consistent with this, both BMYB and the MuvB core bound to DNA probes derived from a target promoter, Cyclin B2, in a manner dependent on the presence of an intact CHR element in this promoter [38]. Similarly, mutation of MYB binding sites in the survivin (BIRC5) promoter disrupted both LIN9 and BMYB binding [57]. The cooperative binding of BMYB and the MuvB core appears to be independent of p130 and E2F4 and the DREAM complex. In cells with little or no DREAM complex, such as rapidly growing embryonic stem cells or HPV E7 expressing cervical cancer cell lines, the BMYB-MuvB complex undergoes cell cycle dependent binding to the promoters of G2/M genes during S phase [52, 53].

Loss of BMYB or MuvB leads to G2/M defects

BMYB has been considered a classical, DNA sequence specific, transcription factor. Overexpression of BMYB will activate a promoter reporter construct containing MYB binding sites or promoters from a late cell cycle gene (reviewed in [60, 61]). Furthermore, loss of BMYB results in lower levels of late cell cycle or mitotic genes [53, 62]. Importantly, the transactivation activity of BMYB is increased upon phosphorylation by cyclin A–CDK2 [63-66]. Accordingly, mutants of BMYB lacking the cyclin A–CDK2 phosphorylation sites were unable to activate transcription [67, 68]. Genetic studies in *Drosophila* and zebra fish also strongly support roles for BMYB in activating mitotic gene expression during G2/M [32, 69-71] and in maintaining chromosomal stability during mitosis[72-74].

There is an apparent paradox in the role of the MuvB core in the context of its interaction with the BMYB transcription factor. Although the MuvB core is required for p130-mediated repression of E2F dependent genes during quiescence [24], it is also required for BMYB dependent transactivation of the late cell cycle G2/M genes [53, 59, 75]. Notably, loss of Myb in fly led to reduced mitotic gene expression as well as adult lethality that can be suppressed by mutations in MuvB or expression of mammalian BMYB [16, 767771]. Similarly, in mammalian cells, mutation or decreased expression of BMYB or MuvB core components results in decreased levels of G2/M genes accompanied by a mitotic arrest. For example, conditional knockout of *Lin9* in mice leads to embryonic lethality, with cells entering S phase with normal kinetics but unable to complete mitosis [34]. In cell lines, knockdown of MuvB components results in reduced expression of late cell cycle genes including cyclin B1 (*CCNB1*), *CDK1*, the ubiquitin conjugating enzyme E2C (*UBE2C*, also known as UBCH10), centromeric protein E (*CENPE*) and polo-like kinase 1 (*PLK1*) with little effect on expression levels of early cell cycle genes, such as *CDC6* and ribonucleotide reductase, M1 subunit (*RRM1*) [25, 53, 75]. Similarly, knockdown of LIN9 or BMYB results in mitotic arrest in F9 embryonal carcinoma cells accompanied by decreased levels of B-type cyclins and survivin (encoded by *BIRC5*) [57]. Depletion of LIN54 also resulted in decreased levels of late cell cycle genes, culminating in cytokinesis defects and mitotic arrest [78]. Expression of a mutant form of LIN54 containing point substitutions in the DNA-binding, cysteine rich (CXC), domain of LIN54, inhibited cell cycle progression and decreased expression of some G2/M genes [39, 79]. These results are consistent with the model that BMYB cooperates with the MuvB core to bind to late cell cycle gene promoters and activate their transcription.

This model does not fully explain how late cell cycle gene expression is regulated. For instance, although phosphorylation of BMYB is required for its transactivation activity, it is also required for its ubiquitination and proteasome-mediated degradation (Figure 3A)[53, 80]. Although the presence of phosphorylated BMYB on the *PLK1* promoter correlates with the start of *PLK1* mRNA expression, there is little BMYB remaining at the point in G2 when maximal levels of *PLK1* mRNA were detected [53]. Recent studies now suggest that an additional transcription factor, FOXM1, cooperates with BMYB and the MuvB core to transactivate the late cell cycle genes [53, 81, 82].

MuvB sequentially recruits BMYB and FoxM1

Several independent lines of evidence in the literature have suggested a potential link between BMYB and FOXM1. The two transcription factors have several target genes in common, including *CCNB1*, *PLK1* and Aurora kinase A (*AURKA*) that are required for progression into mitosis [75, 83, 84]. BMYB and FOXM1 also share common modalities of regulation; their levels are repressed in G0 by the DREAM complex and increased during S phase [24, 58, 85], and they become activated as a result of multi-site phosphorylation due, at least in part, to cyclin A–CDK2 [68, 86]. Both BMYB and FOXM1 undergo cell cycle dependent, ubiquitin-mediated, proteasome degradation. BMYB degradation during G2 requires a cullin 1 (CUL1) dependent E3 ligase activity [80] while FOXM1 degradation occurs later during mitosis in an anaphase promoting complex or cyclosome (APC/C)–cadherin 1(CDH1) dependent manner [87]. In addition, remarkably similar phenotypes have been observed with the loss of FOXM1, BMYB or MuvB. For example, loss of FOXM1 results in

delayed entry into mitosis along with abnormal mitosis and cytokinesis [83]. Knockout of *Foxm1* in mice results in a block to proliferation with defects in mitosis [84]. FOXM1 may also be required to prevent DNA re-replication, particularly in mouse hepatocytes and cardiomyocytes [88].

One of the first demonstrations of a connection between MYB and FOXM1 came from a network analysis aimed at identifying master regulators of B lymphocyte proliferation. This study demonstrated a synergistic activity between MYB and FOXM1 and also showed that the expression of FOXM1 was dependent on MYB [89]. Although MYB is preferentially expressed in cells of the hematopoietic lineage, it is not known if MYB, like BMYB, can bind to the MuvB core [90]. In the same year, another study implicated BMYB in the regulation of FOXM1 expression in embryonic stem cells [91].

More recent studies have strengthened the link between BMYB and FOXM1. A ChIP-sequencing effort for BMYB and MuvB found *de novo* enrichment for MYB, FOXM1 and CHR binding sites in late cell cycle genes suggesting the possibility of co-regulation by all three factors [53]. RNAi knockdown of BMYB, MuvB, or FOXM1 reduced late cell cycle gene expression resulting in delayed cell cycle progression [53, 83]. Conditional knockout of BMYB reduced late cell cycle gene expression and eliminated LIN9 and FOXM1 binding to their promoters [81]. Notably, the combined RNAi-knockdown of BMYB, FOXM1 and MuvB revealed no additive effect on late cell cycle gene expression suggesting that these three factors act through a common pathway [53]. In addition, the MuvB core bound to the FOXM1 and was required for the recruitment of FOXM1 to promoters [53, 82]. Strong evidence supporting cooperative binding between BMYB and FOXM1 was provided by the observation that mutation of the MYB binding site in the *BIRC5* promoter eliminated FOXM1 and LIN9 recruitment as well as BMYB binding and led to reduced peak gene expression during G2 and mitosis [81]. This complements the observation that BMYB, in addition to the MuvB core, was also required for FOXM1 binding to promoters [53, 81].

FOXM1 appears to act downstream of BMYB, with MuvB serving as a bridge linking these two factors. For example, knockdown of BMYB or MuvB reduced binding of FOXM1 to late cell cycle promoters, whereas knockdown of FOXM1 did not affect BMYB or MuvB binding to each other or to late cell cycle promoters [53]. In addition, the MuvB core first binds to BMYB during S phase and then to FOXM1 during G2. However, binding of BMYB and FOXM1 to the MuvB core or to late cell cycle promoters does not appear to be mutually exclusive. ChIP-ReChIP experiments revealed that FOXM1 can bind to same promoter simultaneously with BMYB and the MuvB core [53] (Figure 3A).

How might BMYB and the MuvB core aid in the recruitment of FOXM1 to promoters? The binding of MuvB and BMYB to their cognate target sequences may increase target specificity for FOXM1 DNA binding. This might explain why the BMYB, FOXM1 and CHR binding elements are co-enriched in several mitotic gene promoters [53]. Interestingly, the FOXM1 DNA binding domain has an unusually low affinity for its consensus sequence TAAACA [92]; hence FOXM1 may require cooperative binding with BMYB and MuvB to be specifically recruited to its target promoters. In support of this model, a recent report demonstrates that the CHR element is required for FOXM1 binding to the promoters of

genes expressed late in the cell cycle [82]. Thus, similar to the requirement for MYB binding sites, FOXM1 also requires CHR sites for binding to late cell cycle promoters [81, 82]. Although BMYB and MuvB recruit FOXM1 to the late cell cycle promoters, full activation of its transcriptional activity requires PLK1 dependent phosphorylation [93].

The transcriptional role of BMYB in higher eukaryotes is still unclear, thus its function along with the MuvB core is still to be fully established. Recruitment of FOXM1 to BMYB-MuvB may enable BMYB degradation and thereby relieve MuvB-mediated repression of the late cell cycle genes (Figure 3A and 3B). In an alternative scenario, BMYB and FOXM1 may cooperate in a feed forward loop to activate gene expression. However, this scenario does not explain why BMYB is degraded well before the start of mitosis. Perhaps, in a manner similar to MYC, transactivation activity and ubiquitin-mediated degradation of BMYB are tightly linked [94].

DREAM disruption in cancer

Changes in cell cycle gene levels are the most frequently observed differences when expression profiles of normal and cancer tissue samples are compared [95]. Increased levels of cell cycle genes probably reflect, at least in part, tumours containing a higher fraction of proliferating cells and a lower fraction of terminally differentiated cells compared to normal tissue [96]. However, not all tumours have a high fraction of proliferating cells and not all cell cycle genes are over-expressed in tumours, indicating that for at least some tumours, there may be de-regulation of specific cell cycle genes. Not surprisingly given their central role in promoting late cell cycle gene expression, BMYB and FOXM1, and some of their gene targets including *BUB1*, *PLK1*, *AURKA* and *TOP2A* are frequently over-expressed in tumours and form part of the proliferation signature characteristic of high-grade tumours with poor prognosis [1, 97]. For example, high levels of BMYB (*MYBL2*) are associated with poor prognosis in breast cancer [98]. Notably, *MYBL2* is a component of the breast cancer gene expression signature adapted into the Oncotype Dx clinical biomarker test [99]. This 21-gene panel is widely used to evaluate the risk of recurrence in oestrogen receptor α (ERα, encoded by *ESR1*) positive, lymph node negative, breast cancer. Oncotype Dx also contains a group of BMYB and FOXM1 dependent late cell cycle genes, including *CCNB1*, *AURKA* and *BIRC5*. *MYBL2* is also a component of the 12-gene panel test for colon cancer recurrence although in this test elevated levels of *MYBL2* are predictive of improved survival [100]. In addition, LIN9 is one of the 70 genes that comprise the Mammaprint breast cancer profile used to predict risk for metastasis [101].

Even though high BMYB levels are typically associated with poor outcomes, low levels of BMYB have also been associated with cancer. Zebra fish with mutations in BMYB had mitotic defects as well as an increased risk for developing cancer [74]. Mice haplo-insufficient for *Mybl2* develop a variety of myeloid disorders including a myelodysplastic syndrome (MDS) and myeloid leukaemia [102, 103]. *MYBL2* is present in the 20q locus that is frequently deleted in human MDS [104, 105]. Although the precise mechanism by which low levels of BMYB expression translates into abnormal myeloid cell proliferation is unclear, it is likely to involve BMYB's role in regulating cell cycle dependent gene expression.

Increased expression of FOXM1 has been associated with poor outcomes in many types of cancers. FOXM1 overexpression is correlated with poor prognosis in glioblastoma [106], prostate [107], lung [108], oesophagus [109], pancreas [110], breast [111] and ovarian [112] cancers. Interestingly, FOXM1 is part of the chromosomal instability 70 (CIN70) and CIN25 gene signatures characteristic of aneuploid tumours [113]. Although BMYB is not part of the CIN signatures, several genes regulated by the BMYB-MuvB complex are over-represented in the CIN70 and CIN25 signatures [53, 113].

It is not known if BMYB and FOXM1 overexpression reflects specific de-regulation due to oncogenic mutations and copy number alterations or is due to their central role in controlling late cell cycle gene expression in rapidly growing tumours. Overexpression of BMYB and FOXM1 could potentially alter the balance between DREAM and BMYB-MuvB complexes in a cell. BMYB and FOXM1 levels may be high in cells in which the DREAM function is compromised though the loss of p130 or p107 [24], DYRK1A [29], LATS1 or LATS2 [43]. It has been reported that BMYB overexpression can rescue senescence induced by an activated RAS oncogene in rodent cells [114]. Alternatively, oncogenic mutations that enhance entry into quiescence could enhance tumor survival. For example, DYRK1B is frequently amplified and over-expressed in high-grade serous ovarian carcinoma [115, 116]. It is possible that high levels of DYRK1B could enhance DREAM formation by phosphorylation of S28-LIN52 and enable tumors to escape from the effects of cytotoxic chemotherapy and other stresses.

The balance between the DREAM and BMYB-MuvB complexes can be influenced by p53. Recent studies have demonstrated that activation of p53 in cells treated with DNA damaging agents leads to increased p130-DREAM occupancy and decreased BMYB binding at late cell cycle gene promoters [117, 118]. This p53-mediated shift in favour of DREAM is dependent on p21[117, 118]. The p53-dependent induction of p21 can inhibit cyclin A–CDK2 dependent activation of BMYB and FOXM1 and reduce p130 phosphorylation, thereby shifting the balance from BMYB-MuvB to DREAM (Figure 3B). The late cell cycle G2/M genes with CHR elements in their promoters, including *CDK1*, *CDC25C* and *CCNB1*, appear to be regulated by p53 mediated reactivation of the DREAM complex [117]. It is possible that when DNA damaged cells undergo a G2/M arrest, BMYB-MuvB is replaced by DREAM on late cell cycle promoters [117, 119]. Indeed, re-entry into the cell cycle and progression through M phase after DNA damage requires re-expression of BMYB suggesting the possibility that DREAM was bound to early promoters and subsequently became de-activated to enable expression of BMYB [117]. In this context, it will be important to determine if DREAM-mediated repression contributes to the tumour suppressive function of p53.

Conclusions and perspectives

The MuvB core provides an integrative control point for cell cycle dependent gene expression. The MuvB core contains five proteins, LIN9, LIN37, LIN52, LIN54 and RBBP4 that remain together throughout the cell cycle and alternately bind to at least 3 different transcription factors at distinct times of the cell cycle. The MuvB core binds to p130 and the E2F4–DP1 transcription factor during quiescence to repress the expression of hundreds of

cell cycle dependent genes. Upon entry into the cell cycle, p130 dissociates from the MuvB core and from E2F promoters thereby permitting the activating E2Fs to transactivate the early (G1/S) cell cycle genes. The MuvB core sequentially recruits BMYB during S phase and then FOXM1 during G2 to promote the expression of the late (G2/M) cell cycle genes. The switch from DREAM to BMYB-MuvB is highly regulated. At a minimum, the CDKs disrupt the interaction of p130 with the MuvB core and promote BMYB-MuvB activation. Opposing this, DYRK1A, and perhaps DYRK1B, have an essential role in enabling binding of p130 to the MuvB core to form the DREAM complex and permit exit from the cell cycle into quiescence.

The balance between the quiescent DREAM complex and the proliferative phase BMYB-MuvB-FOXM1 complex is frequently perturbed in cancer. Deregulated cyclin and CDK activity will disrupt DREAM as well as RB resulting in de-repression of the E2F dependent cell cycle genes and activation of the late cell cycle genes. Loss of E2F6 [120] and the atypical E2F7 [121] and E2F8 [122-124] repressor E2Fs, which oppose activator E2F induced transcription, may provide yet another means of increasing early cell cycle gene expression. Alternatively, decreased levels of BMYB due to gene loss or increased DREAM formation due to DYRK1A-B overexpression may enable cancer cells to exit the cell cycle and remain dormant. Although RB may serve a distinct role in repressing activator E2F-dependent transcription, it is not clear if the loss of RB and perturbation of DREAM can be distinguished in cancer. This leaves open the question of whether there are separate roles for RB and DREAM during quiescence and oncogene-induced senescence and in cancers.

Beyond quiescence and senescence, there are many unanswered questions regarding the role of the MuvB core and the DREAM and BMYB–MuvB–FOXM1 complexes in cell cycle regulation. The structure of the MuvB core is unknown. Although pS28-LIN52 is required for the interaction of MuvB with p130, additional requirements for the interaction of the MuvB core with BMYB and FOXM1 are still unknown. It is also unclear whether the MuvB core contributes directly to promoter activation or repression or if additional factors, such as chromatin remodelling enzymes or modified histones, cooperate with the MuvB core to control cell cycle gene expression in mammalian cells as suggested by genetic studies in worm and fly. Perhaps enhanced biochemistry techniques combining cross-linking with mass-spectrometry will identify additional specific binding factors [125]. Alternatively, reconstitution of the MuvB core complex in vitro using chromatinized templates may address these important questions.

It is also not clear whether the MuvB core regulates gene expression beyond the cell cycle. Studies in fly have revealed a role for MMB-dREAM in olfactory receptor gene expression [126], programmed cell death genes in neural tissues [127], and repression of germ line gene expression in intestinal tissues [128]. Perhaps isolation of the DREAM and BMYB-MuvB complexes from distinct mammalian tissues will reveal functions in addition to cell cycle control [129, 130]. It is not known how the fly Myb-MuvB complex contributes to chorion gene amplification[14] and it remains to be determined if and how Myb and the MuvB core contribute to DNA replication in fly or mammalian cells. Interestingly, while aberrant DNA replication has been observed in *MYBL2* knockout MEFs [102], it is unclear if this is the outcome of a direct role of BMYB in DNA replication. There is some evidence that

signalling from DNA damage influences the balance between DREAM and MYB-MuvB complexes[117], although the molecular details remain unknown. A related question is whether the timing of BMYB degradation is regulated by specific events such as the completion of DNA synthesis or signalling from DNA damage checkpoints. Identifying the E3 ubiquitin ligase responsible for targeting BMYB to the proteasome could provide insight into how a cell recognizes the end of S phase and start of G2.

In terms of cancer research, it will be important to determine if quiescence and the DREAM complex play an important role in tumour biology. If so, for example, does DYRK1A or DYRK1B function as tumour suppressors? Moreover, in a similar context, does the Hippo tumour suppressor pathway regulate LATS1 and LATS2 activity in terms of the phosphorylation of DYRK1A and DREAM assembly? Identifying tumours with loss of DYRK1A or LATS may reveal a role for quiescence in tumour suppression. At a basic level we also need to understand how oncogenic perturbations in cell cycle regulators impact DREAM and the MuvB core and if they differentially perturb RB and the DREAM complex. Given the frequency of FOXM1 overexpression in cancer, it is important to determine whether its contribution to poor prognosis is due to its ability to promote late cell cycle gene expression or whether this reflects additional activities. Lastly, despite the challenges in targeting oncogenic transcription factors, BMYB, FOXM1 and MuvB all undergo extensive post-translational modifications that could render them susceptible to novel therapeutics.

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Box 1. RB mediated cell cycle progression

The control of the cell cycle by RB, the product of the retinoblastoma tumour suppressor gene (*RB1*), has long been recognized. The central tenet has been that hypo-phosphorylated RB binds to E2F transcription factors during G1 and inhibits cell cycle dependent, E2F-mediated, gene expression. RB preferentially binds to the activating E2F transcription factors, E2F1, E2F2 and E2F3, with their dimerization partners DP1, DP2 and DP3 and represses their activity. The activating E2Fs promote expression of genes required for DNA synthesis during G1/S. Phosphorylation by cyclin D–cyclin dependent kinase 4 (CDK4) and cyclin E–CDK2 releases RB from E2F transcription factors and thereby permits cell cycle gene expression. However, the control of entry into S phase by RB does not fully account for how gene expression is regulated throughout the cell cycle.
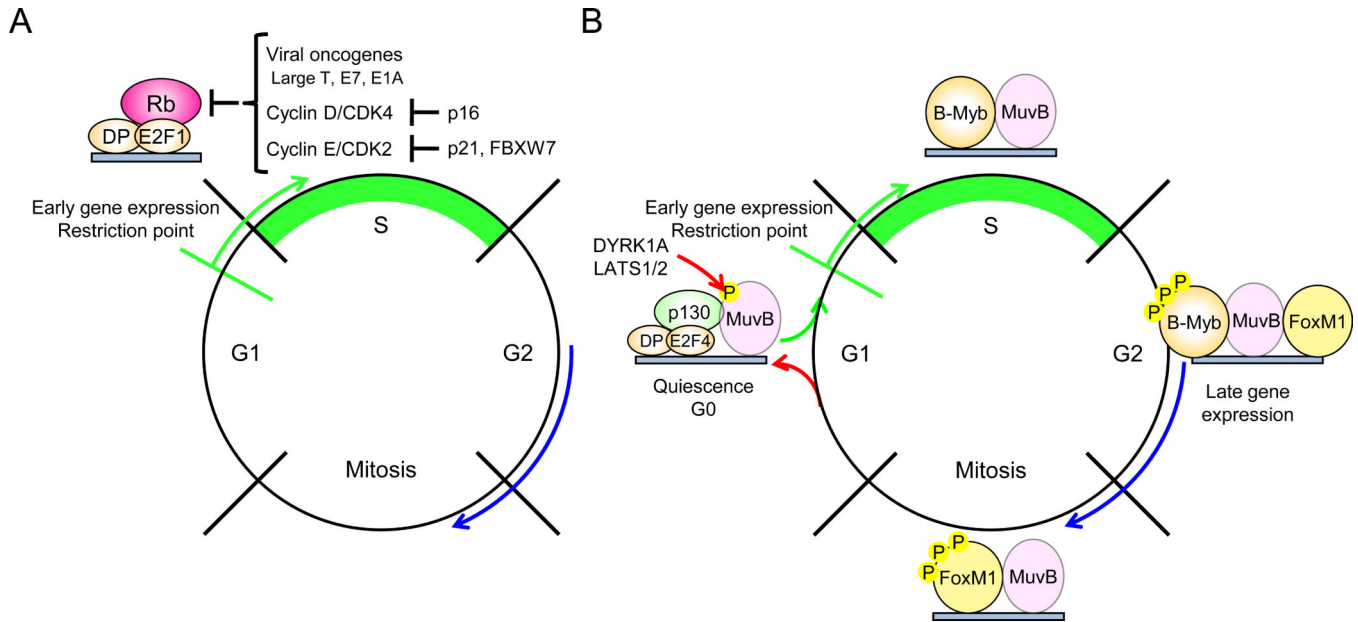
Figure 1. Differential cell cycle control by RB and DREAM

A. RB mediated repression of the activator E2Fs is relieved after cells pass the restriction point. Cyclins D and E in complex with cyclin dependent kinases (CDKs) relieve RB-mediated repression of E2F. The activator E2Fs including E2F1, E2F2 and E2F3 contribute to expression of the early cell cycle genes during the G1 to S phase transition (green arrow). RB may restrict E2F dependent gene expression during DNA damage signalling or oncogene-induced senescence. The classic model of the RB-E2F pathway depicted here does not account for the expression of G2/M late cell cycle genes (blue arrow) during G2/M. Perturbations in RB control of E2F activity occur in most cancer types and include inactivating mutations of the *RB* gene itself, increased CDK activity due to overexpression of cyclins D and E or loss of FBXW7, decreased expression of CDK inhibitors such as p16^{INK4a} and p21, or expression of viral cyclins such as KSHV viral cyclin that binds to cellular CDKs and renders them resistant to inhibition by CDK inhibitors [131, 132]. In addition, the viral oncoproteins, papilloma virus E7, polyomavirus large T antigen or adenovirus E1A can bind to RB and dissociate it from E2F.

B. The central role of the MuvB core in binding and directing key transcription factors to the promoters of cell cycle genes during various cell cycle phases. MuvB binds p130-E2F4-DP to form DREAM in G0 and repress all cell cycle dependent gene expression. Association of the MuvB core with p130-E2F4 is dependent on DYRK1A-mediated phosphorylation of the MuvB subunit, LIN52. The MuvB core sequentially recruits BMYB during S phase and then FOXM1 to the promoters of the G2/M genes. In all three instances, the MuvB core is essential for targeting the complexes to specific sets of cell cycle gene promoters.
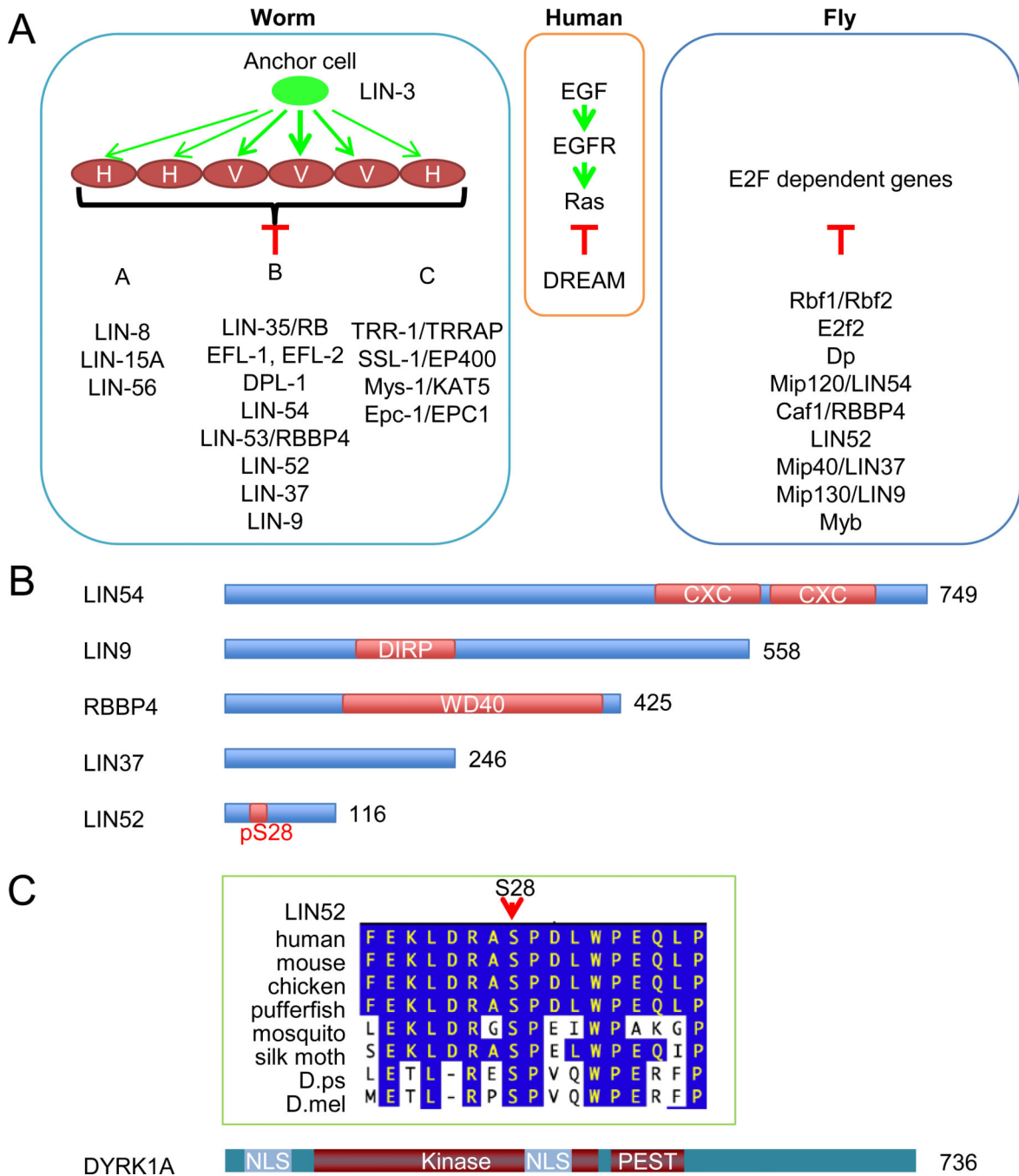
Figure 2. Common themes of repression connect the Muv genes and their homologs
**A.** Functional antagonism between the MuvB and RAS pathways is evolutionarily
conserved. The pathway for development of the vulva in Caenorhabditis elegans is initiated
through LIN-3, an epidermal growth factor (EGF)-like ligand. Cells in closer proximity to
the anchor cell receive greater amounts of the LIN-3 signal (thick green arrow), and activate
a RAS signalling cascade to ultimately acquire a vulval fate (V). Cells farther away receive
lesser amounts of the LIN-3 signal (thin green arrows) and develop the hypodermal fate (H).
Increased levels of LIN-3 are required to overcome the repressive effects of the synthetic

multi-vulval (synMuv) class of genes (A, B and C) on RAS signalling. Notably, homologs of genes in the RB-E2F pathway feature predominantly as class B (MuvB) genes. In Drosophila melanogaster, RB-E2F homologs as well as homologs of several synMuvB genes are required to repress the group E genes, which show sex- and differentiation-specific expression patterns.

B. Domains of human MuvB core subunits. Schematic of domains and residue number present in subunits of the MuvB core complex as revealed by Entrez Conserved Domain (CD)-search. The cysteine rich motif domain (CXC) may represent the specific DNA-binding module of LIN54. LIN9 contains a domain in RB-related pathway (DIRP), present in many eukaryotic genomes and its function is unknown. Retinoblastoma binding protein 4 (RBBP4) and its WD40 domain may recruit other proteins or modified histones.

C. The amino acid sequence surrounding Serine 28 (S28) residue in LIN52 is highly conserved across several eukaryotic genomes. Domains present in DYRK1A, the kinase responsible for phosphorylating LIN52 at S28 are shown.
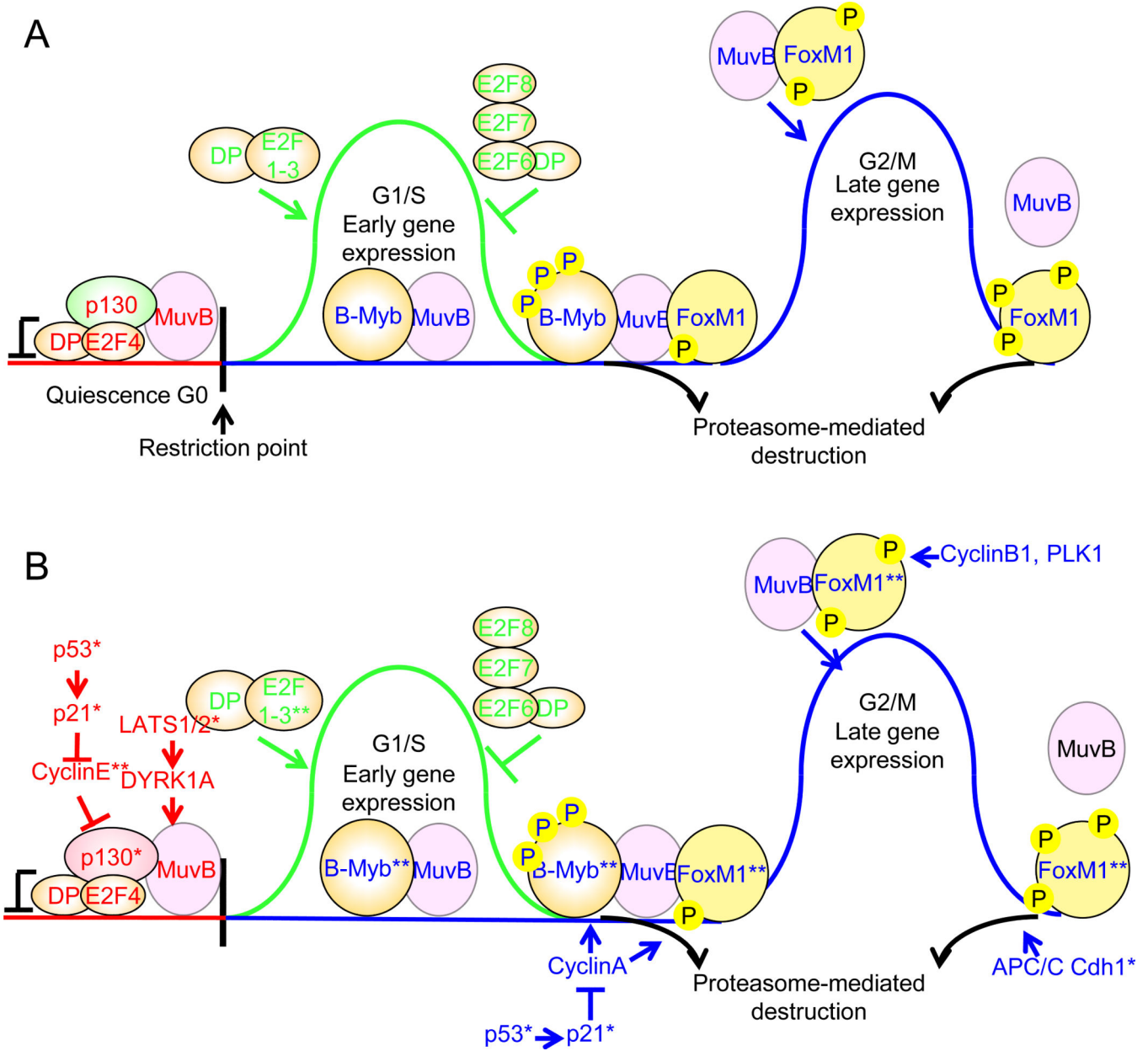
Figure 3. Tipping the balance from quiescence to proliferation in cancer
A. Differential regulation of early and late cell cycle gene expression. At least two major peaks of cell cycle gene expression can be discerned during the cell cycle; occurring during G1/S (green line) and G2/M (blue line). The DREAM complex represses both early and late cell cycle gene expression during quiescence (red line). Activator E2Fs (in green) are required for the expression of G1/S genes, while the MuvB core complex, BMYB and FOXM1 (in blue) are required for the expression of G2/M late genes. BMYB and FOXM1 undergo phosphorylation and ubiquitin dependent destruction by the proteasome during S to G2 phase and M to G1 phase transitions, respectively. Repressor E2Fs including E2F6-DP, E2F7 and E2F8 contribute to repression of early or late cell cycle genes, particularly after DNA damage [120, 122, 133-135].

B. Loss of DREAM and gain in B-Myb, MuvB and FoxM1 in cancer. Factors over-expressed or amplified (**) or lost (*) in cancers are indicated. The net effect results in loss of DREAM and increased activity of BMYB-MuvB-FOXM1 driving cells out of quiescence and to proliferation.

Table 1

MuvB containing complexes and their orthologous components in human, fly and worm.

Human (MuvB containing complexes[24])		Fly (MMB/dREAM[15, 17])	Worm (DRM[20])	SynMuvB	Function
	RBL1 (p107) RBL2 (p130)	Rbf1 Rbf2	lin-35	Yes	E2F binding
	E2F4 E2F5	E2f2	efl-1	Yes	sequence specific transcription factor
	DP1 DP2 DP3	Dp	dpl-1	Yes	dimerization partner of E2F
	LIN9 (BARA)	Mip130 (twit)	lin-9	Yes	unknown
	LIN54	Mip120	lin-54	Yes	CHR binding [39]
	LIN37	Mip40	lin-37	Yes	Unknown
	LIN52	Lin52	lin-52	Yes	interaction with p130 [29]
	RBBP4 (RbAp48)	Caf1 (p55)	lin-53	Yes	chromatin binding
	MYBL2 (BMYB)	Myb	?	?	sequence specific transcription factor
	FOXM1	?	?	?	sequence specific transcription factor
	-	Rpd3	-	hda-1, Yes	Histone deacetylase
	-	L(3)mbt	-	lin-61, Yes	Me-histone binder

(Left-margin bracket labels: **DREAM**, **BMYB-MuvB-F**)

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Table 2

Sequence specific DNA elements bound by mammalian MuvB-containing complexes.

DNA Sequence Element	Primary Sequence Consensus	Transcription Factor	Examples of Target Genes
Cell cycle genes Homology Region (CHR)[38, 39]	5'-TTTGAA-3'	LIN54 / MuvB core	*CDC2, PLK1, AURKB, CKS1*
Downstream Repression Site (DRS)[40]	5'-GGAAA-3'	p130/p107, E2F	*MYBL2*
Cell cycle Dependent Element (CDE)[37]	5'-E2F site-NNNNCHR-3'	E2F?	*CDC2, AURKB, CKS1*
E2F site [136, 137]	5'-TTTSSCGC-3' (S=G or C)	Heterodimers of E2F1/2/3 or E2F4/5 with DP1/2	*PCNA, CDC6, POLA, E2F1*
Myb-Recognition element (MRE)[81, 138, 139]	5'-YAACKG-3' (K=G or T)	AMYB, BMYB and MYB	*CCNB1, CDC2, BIRC5*
FOXM1 binding sequence[140]	5'-TAAACA-3'	FOXM1	*CKS1, AURKB, CENPA*